EVIDENCE OF NATURAL HYBRIDIZATION BETWEEN MIMULUS RINGENS AND MIMULUS ALATUS (SCROPHULARIACEAE)[1]

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A population of *Mimulus* that consisted of plants typical of *M. ringens* L. and *M. alatus* Ait. as well as plants that exhibited intermediate morphological characteristics was located along the Patapsco River in Baltimore Co., Maryland. A mass collection of the population was made and morphological and chemical studies were initiated to determine whether or not the populational variation was the result of hybridization between the above two taxa.

Mimulus ringens and *M. alatus* are the only representatives of *Mimulus* sect. *Mimulus* (sect. *Eumimulus* of Gray). This section is characterized as a group of erect, glabrous perennials with pinnately veined leaves and leafy racemes. The flowers are usually violet or violet-purple, but occasional white forms are observed. The more common *M. ringens* has clasping or sessile leaf bases, wingless stems, peduncles 3-6 cm. long, and calyx teeth that are usually longer than 2 mm. *Mimulus alatus* has petiolate leaves, winged stems, peduncles less than 1 cm. long, and calyx teeth that are usually shorter than 2 mm.

According to Pennell (1935) the two species are sympatric over most of the eastern United States. He described the habitat of both species as "stream-banks, swales, and swamps, especially where alluvial or calcareous"; however, the authors, while gathering materials of the two species in the Knoxville, Tennessee area, observed that *Mimulus ringens* occurs in open, marshy areas while *M. alatus* occupies shaded stream banks. Whether this generalization holds over the entire range of the two species will require further investigation.

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MATERIALS AND METHODS

Specimens used in this study were collected along the north side of the Patapsco River just west of Gun Road in Baltimore Co., Maryland (*Mimulus ringens* — *Windler* 4083, *M. alatus* — *Windler* 4082 and putative hybrids — *Windler* 4081) and in Knox Co., Tennessee, in open, marshy areas 0.4 mile east of US 129 on the south side of Cherokee Blvd. (*M. ringens* — *Wofford & Windler* 5000) and in the woods along a shaded stream bank 4.1 miles south of the Tennessee River on the east side of US 129 (*M. alatus* — *Wofford & Windler* 5001). Vouchers are deposited in the University of Tennessee Herbarium, Knoxville (TENN). Although this study centers on the Patapsco River population, specimens from pure populations of *M. ringens* and *M. alatus* from the Knoxville area were examined for purposes of comparison.

The Patapsco River population consisted of approximately fifty individuals of which a very high percentage (ca. 40%) were designated as putative hybrids. Of the remaining plants, ca. 35% were designated as *Mimulus ringens* and ca. 25% as *M. alatus*. The plants were randomly scattered in a partially shaded drainage depression that did not contain water at that time but appeared to collect and retain water for short periods during rains. This could be considered a fairly intermediate habitat between the open, marshy habitat of *M. ringens* and the shaded stream bank habitat of *M. alatus* observed in the Knoxville, Tennessee area.

A scatter diagram of the Patapsco River population (Fig. 1) was constructed in order to obtain a visual representation of the populational variability. Because leaf base shape and winging of the stem are difficult characters to quantify, peduncle length and calyx lobe length were used as the coordinates of the diagram. Measurements were made on flowers at anthesis.

Pollen was obtained from anthers of flowers at anthesis and stained with aniline blue in lactophenol as an indicator

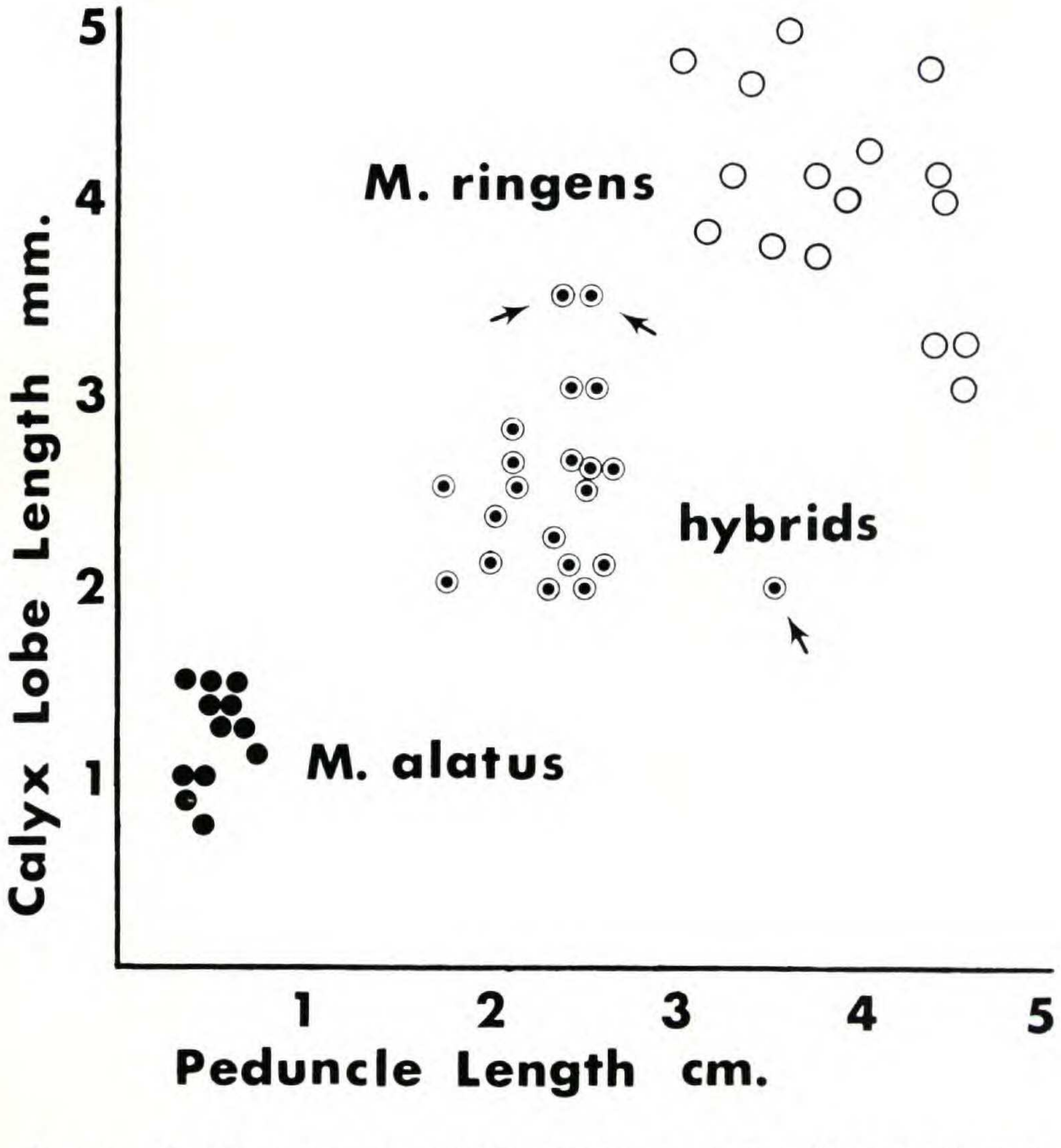

Fig. 1. Scatter diagram of the Patapsco River, Maryland population of *Mimulus*.

of pollen viability. The mean, standard deviation and range were determined for *Mimulus ringens, M. alatus,* and putative hybrids from the Patapsco River population and for *M. ringens* and *M. alatus* from pure populations (Table 1). The means were then analysed statistically by the Duncan's new multiple range test modified for unequal numbers of observations (Steel & Torrie, 1960) to determine if there were significant differences in percentages of pollen viability.

All plants used in this study were analysed for flavonoid chemical constituents (Fig. 2, Table 2) following the methods of Mabry, Markham, and Thomas (1970). Mid-stem leaf material was extracted overnight in 85% methanol, the extract was spotted on Whatman 3MM chromatographic paper and chromatograms were developed in solvent systems of tertiary butanol:glacial acetic acid:water (3:1:1) for the first dimension and 15% glacial acetic acid for the second dimension. When sufficient quantities could be isolated, compounds were analysed by ultraviolet spectroscopy and were hydrolysed by refluxing at 100° C for one hour (3 hours for compound 2) in 6% HCl. Aglycones and sugar residues were separated by partitioning in ethyl acetate and water, the aglycones moving into the ethyl acetate and the sugars into the water. Aglycones were

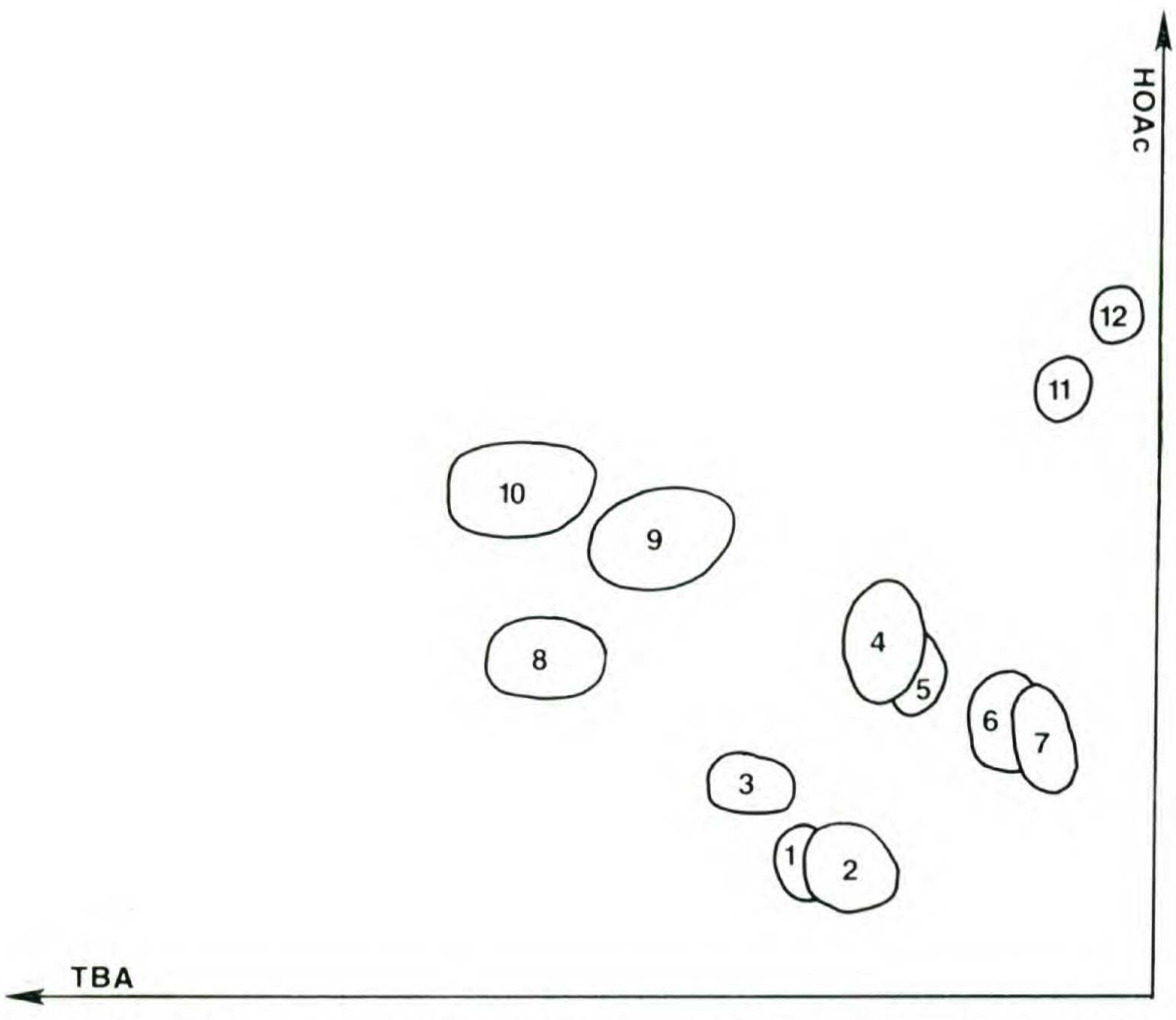

Fig. 2. Composite chromatographic profile of flavonoids in *Mimulus* (Sect. *Mimulus*).

then rechromatographed and analysed by standard procedures. Trimethylsilyl ether derivatives of the sugars were prepared and analysed by comparison to known standards on a Bendix 3600 gas chromatograph equipped with a 6 ft. × 0.25 in. column packed with acid washed silanized chromosorb W coated with 3% SE-52 and run at a temperature of 180° C.

RESULT AND CONCLUSIONS

The scatter diagram (Fig. 1) separates the individuals into three groups. Those at the lower left are most like *Mimulus alatus,* those at the upper right are most like *M. ringens* and those in the center are putative hybrids. The putative hybrids, while fairly closely clustered, exhibit some variability in the direction of the *M. ringens* individuals. This relatively tight clustering would suggest that most are probably F_1 hybrids, but the existence of three individuals somewhat separated from the others (Fig. 1, arrows) could indicate that backcrossing to *M. ringens* has occurred. The variability observed in the *M. ringens* individuals could simply be the result of phenotypic plasticity as is commonly observed in outcrossing species. However, the existence of putative backcross individuals suggests the additional possibility that the morphological variability in *M. ringens* could be the result of introgression. The tight clustering of the *M. alatus* individuals and their clear spacial separation on the diagram from the putative hybrids suggests that there has been little or no backcrossing in the direction of *M. alatus.*

The pollen stainability results are shown in Table 1. The Duncan's new multiple range test showed that pollen stainability in the putative hybrids is significantly lower than that in *Mimulus ringens* and *M. alatus,* but that there are no significant differences among the parental groups tested. If extensive backcrossing (i.e., introgression) were occurring, and assuming that genetic material of one taxon entering the gene pool of another taxon will cause some

Table 1. Pollen Stainability in *Mimulus* (Sect. *Mimulus*)

Taxon	Number of observations	Percent Stainability	St. Dev.	Range
M. alatus (Tenn.)	7	95.71	5.05	85-99
M. alatus (Md.)	10	95.90	4.48	86-100
hybrids (Md.)	16	36.62	8.78	18-49
M. ringens (Md.)	14	94.14	3.99	87-100
M. ringens (Tenn.)	7	97.00	3.26	90-99

meiotic irregularities and hence a higher percentage of inviable pollen, one might expect to observe lower pollen stainability in the parents of the mixed population compared to the parents from pure populations. This does not appear to be an unreasonable assumption in light of the fact that the three individuals indicated on the scatter diagram as possible backcrosses (see also chemical discussion below) have pollen stainabilities of 27%, 34%, and 41% compared to a range of 87-100% among the *M. ringens* individuals. The significantly lower pollen stainability in the putative hybrids, therefore, strongly suggests that hybridization is occurring in the Patapsco River population while no significant differences in pollen stainability among the parental taxa suggest that extensive backcrossing to either parent is not occurring.

A total of 12 phenolic compounds were detected on the chromatograms of the two species and the putative hybrids (Fig. 2). Unfortunately, many of the compounds were very weak and difficult or impossible to identify even after large quantities of leaf material were extracted and chromatographed on columns of Sephadex LH-20. We were unable to obtain any ultraviolet spectral data on compounds 1, 4, 5, 6, 7, and 12, and each of these compounds, therefore, is assumed to be identical in different individuals on

Table 2. Distribution of flavonoids in *Mimulus* (Sect. *Mimulus*)

Taxon	Flavonoid											
	(1)	Luteolin 7-0-glucosylglucuronide (2)	Apigenin 7-0-glycoside (3)	(4)	(5)	(6)	(7)	Quercetin 3-0-glucoside (8)	Quercetin 3-0-rhamnoglucoside (9)	Kaempferol 3-0-rhamnoglucoside (10)	Quercetin 3-0-glycoside (11)	(12)
M. alatus	+	+	+	+	+	+	+	+			+	
hybrids	+	+	+	+	+	+	+	+	+	+	+	+
M. ringens		+	+	+			+	+	+	+		

the basis of Rf values and color characteristics. Spectral data only were obtained for compounds 3 and 11 and they were partially identified as an apigenin 7-0-glycoside and a quercetin 3-0-glycoside, respectively. Spectral data and sugar analyses were obtained for the remaining compounds, which were identified as luteolin 7-0-glucosylglucuronide (2), quercetin 3-0-glucoside (8), quercetin 3-0-rhamnoglucoside (9), and kaempferol 3-0-rhamnoglucoside (10). Of the 12 compounds shown in figure 2, 4 are found

in *Mimulus alatus*, 2 are found in *M. ringens* and 5 are found in both species. All but three of the putative hybrids contain all compounds including one (12) not found in either species (Table 2). This distribution of flavonoids represents a classical case of hybrid complementation in the sense of Alston and Turner (1963) and the individuals with complementary chromatographic profiles, therefore, are probably F_1 hybrids. The three putative hybrids (*Windler* 4081-12, 20 and 21) that do not exhibit complementation are the same ones that were noted earlier as being somewhat separated from the other putative hybrids (Fig. 1, arrows). Their flavonoid profiles are similar to *M. ringens* and they may, as suggested earlier, represent backcross individuals. Levin (1967, 1968), for example, found that backcross individuals of *Phlox* and *Liatris* usually have the chromatographic profile of the recurrent parent and contain none of the compounds of the non-recurrent parent. That the above three plants are indeed backcrosses and not variable *M. ringens* individuals is further substantiated by other data that show that they are fairly well separated from the *M. ringens* plants morphologically, and more importantly they exhibit low percentages of pollen stainability when compared to the *M. ringens* individuals (see pollen stainability results).

SUMMARY

Morphological and chemical evidence as well as pollen stainability data indicate that extensive hybridization is occurring in the Patapsco River population. Furthermore, morphological and chemical data are in agreement that at least limited backcrossing to *Mimulus ringens* is taking place. However, pollen data strongly suggest that backcrossing has not been extensive and, therefore, the morphological variability in *M. ringens* noted in the scatter diagram is probably due to phenotypic plasticity and is not the result of introgression.

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